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Effects of Long-term Exposure to m-Cresol on the Quality of Common Carp (*Cyprinus carpio L.*)

S.I.A. Hasanin and I.M. Fouad

Central Laboratory for Aquaculture Research, Agricultural Research Center, Egypt

Abstract: This investigation was performed to investigate the effect of long-term exposure of common carp (*Cyprinus carpio L.*) to m-cresol pollution. For this purpose fish were exposed to sublethal dose of m-cresol for 90 days. The pathological changes in skin and muscles, residues, muscle yield, chemical composition and organoleptic properties were recorded at the end of this period. The results showed histopathological alterations in the skin and muscles with a significant decline in the total protein, carbohydrate and lipid contents in the flesh of exposed common carp. Residues, bad taste and odor with decreased in muscle yield were observed. Long term exposure to m-cresol pollution affects fish quality and also high loss may occur through the rejection of fish.

Key words: Common carp · m-cresol · Chemical compositions · Organoleptic properties

INTRODUCTION

Fresh fish is a highly perishable product due to its biological composition. It is rich in polyunsaturated fatty acids ω-3 fatty acids, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids] and an excellent source of high quality protein that contains sufficient amounts of most essential amino acids which play an important role in human health and nutrition [1]. So the majority of countries including Egypt express a great effort to increase the fish cultures to fulfill the shortage of protein production. Carp fish, is one of the freshwater fish species which widely cultured all over the world due to its fast growth rate, easy cultivation, high feed efficiency ratio as well as high nutritional value [2]. Recently, the rapid development of chemical industry in Egypt, have been created the environmental pollution. Crude cresols are derived from the heavy oil of coal tar or petroleum, wood and other biogenic materials. It is still used as disinfectants or herbicides. They are also used in the textile industry as cleaning agents, wood and paper mill industries. The presence of creosols pollutants led to many serious hygienic and economic problems [5]. The adverse effects of cresols on aquatic life may be due to its direct toxicity to the aquatic organism or introduce bad taste and odor to edible flesh of fish [3] and may lead to a risk of stomach cancer in the consumers [4].

It was reported that LC_{50} of cresols for fish, was 2.3-29.5, 6.4-24.5 and 4.0-21.2 mg/L for o-cresol, m-cresol and p-cresol, respectively [5]. Exposure to sublethal levels of cresols wastes in common carp (*Cyprinus carpio L.*) induced hyperplasia of epidermal mucous cells as well as degenerative with necrotic changes in muscles, liver and kidneys [6]. Hepatic neoplasms in the mummichog (*Fundulus heteroclitus*) from a creosote-contaminated site were observed [7]. The presence of creosote in wood industries effluents decreased hatching success. Also, the hatched larvae exposed as embryos to creosote exhibited morphological deformities, including scoliosis, pericardial edema and/or ascites [8].

The aim of the present work was to determine the pathological effects as well as the residues, muscles yield, chemical composition and organoleptic properties of common carp (*Cyprinus carpio L.*) after long term exposure to m-cresol.

MATERIALS AND METHODS

Fish: A total number of 120 apparently healthy sexually mature common carp (*Cyprinus carpio L.*) with an average body weight of 282 ± 1.2 g. and both sexes were obtained from the ponds of Central Laboratory for Aquaculture Research during March, 2009. Fish were transferred alive from the ponds to the laboratory and divided into four equal groups.

Corresponding Author: Samya I.A. Hasanin, Department of Fish Processingand Quality Controler, Central Laboratory for Aquaculture Research, Agricultural Research Center, Egypt

Each group was placed in 530 L fiberglass tanks containing dechlorinated tap water and acclimatized for two weeks prior to the experiment with continuous aeration. Pure m-cresol (CH_3 C_6H_4 OH) was purchased from Egyptian Company for Chemicals and Pharmaceuticals, ADWIA.

Experimental Design for Long Term Exposure to m-Cresol: Two main groups of common carp (*Cyprinus carpio L.*), the first one (30 fish) was used as non-exposed control. The second exposed group (90 fish) put into three fiberglass tanks (each contains 30 fish) and exposed to 15 ppm of m-cresol according to El-Tabakh [6] for 90 days. All groups were kept under the same condition including: The physical parameters of water were 7.9±0.5 mg/L dissolved oxygen, 7.2±0.3 pH and 20±2 ºC water temperature. The fish were fed once daily on a commercial fish food at a level of 3% of their body weights according to Eurell *et al.* [9]. Siphoning of 95% of water from the fiberglass tanks were replaced by an equal volume of water containing the same concentration of the toxicant every day. Fish samples were collected at the end experiment. Morphological examination was done as described by Schäperclaus *et al.* [10]. The specimens from caudal muscular regions were taken for histopathological studies. Fillet samples were taken for residue analysis, muscle yield, chemical composition and organoleptic properties after 90 days post exposure.

Histopathological Examinations: Tissue specimens from the skin and muscles of experimental fish were trimmed after 90 days post exposure (PE) and fixed in 10% phosphate buffer formalin solution. Paraffin sections of 5 microns thickness were prepared and stained with Hematoxylin and eosin (H. and E.) and examined microscopically [11].

Residual Analysis: Samples from the skin with muscles of both control and experimented fish were collected for determination of m-cresol residues after 30, 60 and 90 days (PE). According to procedures recommended by AOAC [12] using atomic absorption spectrophotometer.

Fillets Preparation: At the end of experiment 30 common carp from control and 30 from exposed groups were subjected to filleting. In which the head, scales and all fins were removed from fish using a sharp knife. Thereafter, the fish were washed with tap water and then the whole fish was eviscerated and filleted. Fish fillets were obtained from both sides of the fish, along the spine and ribs, each individual was weighed in precision scale (0.1g) and measured (length and height measurements were done in mm). The total fork length, whole body weight and fillets with skin weight of each fish were recorded.

Analytical Techniques for Proximate Chemical Compositions: Homogeneous mixtures of fillets with skin (3-5g) were used for moisture determination by the weight loss after 4 h. at 60 ºC in an assisted air circulation oven, followed by 8 h. at 105ºC [12]. Crude protein (N-6.25) was determined by the microKjeldahl procedure (method 960.52) of the AOAC [12]. Fat was extracted using chloroform and methanol as described by Bligh and Dyer [13] and used for determination of fat content. Ash content was determined at 550 ºC using a muffle furnace (method 923.03) according to AOAC [12]. The total carbohydrates % = 100-(moisture% + total protein %+ fat % + ash %) according to Egan *et al.* [14].

Organoleptic Properties: Samples from m-cresol treated fish were evaluated for appearance, color, texture and overall acceptability after 90 days post exposure as described by Teeny and Miyauchi [15]. Scoring was done according to the following scheme:

Score	Description	Score	Description	Score	Description
10	Ideal	6	Fairly good	2	Poor
9	Excellent	5	Acceptable	1	Very poor
8	Very good	4	Fair	0	Repulsive
7	Good	3	Poorly fair		

Statistical Analysis: Three replicates of each trial were performed to evaluate m-cresol residues in fillets including skin as well as proximate chemical compositions (total protein, fat, ash and carbohydrates) in control and post exposure to m-cresol. Data were analyzed using Analysis of Variance (ANOVA) and means were separated by Duncan at a probability level of < 0.05 [16].

RESULTS

Morphological Changes: Fish in the control group (non treated) revealed normal morphology, while the, exposed fish showed excessive mucus covering the skin with gills. Focal scales loss and erosions of skin after 90 days (Fig.1).

Histopathological Changes: Skin and muscles in the control group (non treated) revealed normal tissues structures (Fig.2 and 3). The skin of treated group showed hyperplasia of epithelial cells and mild dermal

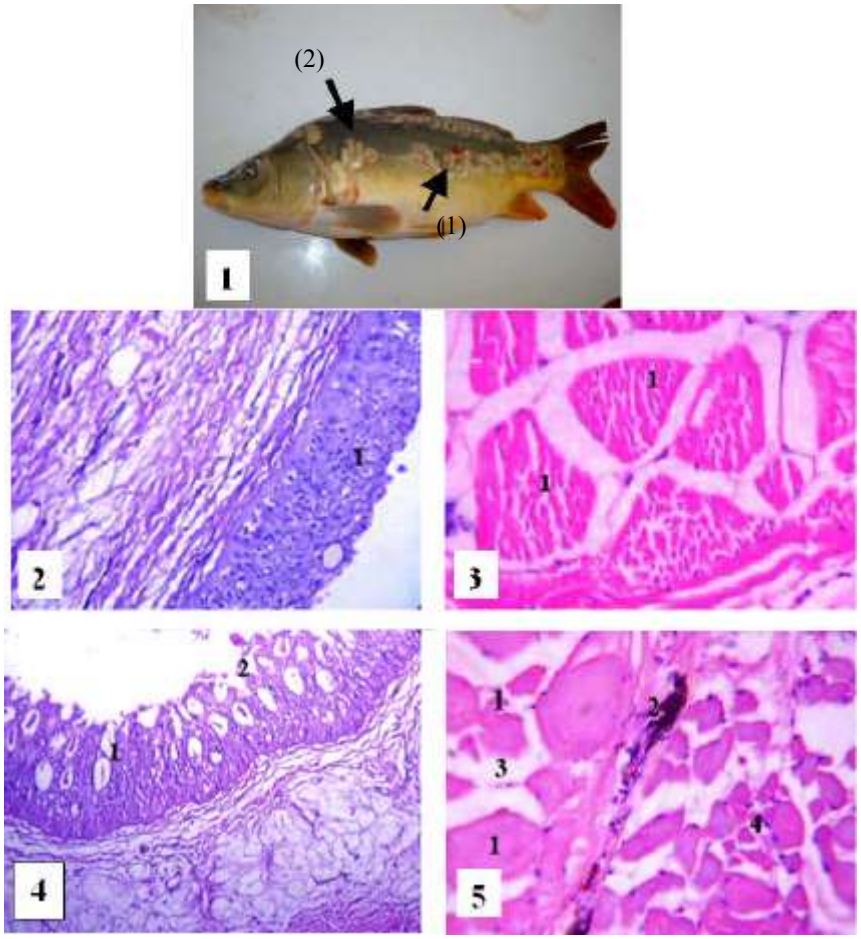

Fig. 1: Common carp exposed to m-cresol after 90 days showing focal erosions[1] and skin darkening[2].
Fig. 2: Non-exposed control common carp skin showing normal epidermis (H. and E., X 250).
Fig. 3: Non-exposed control common carp showing normal muscles bundles (H. and E., X250).
Fig. 4: Skin, of common carp exposed to m-cresol after 90 days, showing hyperactivation of the mucous cells[1] with erosion of epidermis[2] (H. and E., X 250).
Fig. 5: Muscle, of common carp exposed to m-cresol after 90 days, showing hyaline degeneration[1] and melanomacrophages[2] with edema[3] among the necrotic muscles bundles[4] (H. and E., X 250).

edema after 30 days of PE. Hypertrophy of the epidermal mucous cells and erosions (Fig.4). Focal proliferation of melanomacrophages in the dermis were seen after 90 days PE. The muscles suffered focal hyaline degeneration along the period of experiment. Zenker's necrosis, edema and focal proliferation of melanomacrophages were seen among the necrotic muscles bundles after 90 days PE (Fig. 5).

Residues Analysis: Random fish muscle samples were taken post exposure to (15 ppm) m-cresol. Table 1 demonstrats that, m-cresol was accumulated in the muscles of experimental common carp during 90 days (PE). While, it was not detected in control.

Fillets Yield and Chemical Composition: Table 2 shows the mean and range of weight (g), length, height (cm) and yield (%) of common carp exposed to m-cresol for 90 days. The results indicated that, the fillets yield for each treatment were expressed as the total weight of both boneless fillets divided by the total weight of the whole fish in the round. As for fillets weight, length, height and yield, common carp control fillets had the highest values when compared with the common carp exposed to m-cresol which were 108.3 g., 11.3 cm, 7.7 cm and 36.1%, respectively. On the other hand, non edible weight percentages were 64.7, 64.0 and 70.2% after zero and 90 day for control and treated groups by m-cresol, respectively.

Table 1: Residual analysis of m-cresol in common carp muscles during 90 days compared to control

	Time of sampling (days)			
Item	Zero	30	60	90
Control	ND	ND	ND	ND
m-cresol (ppm)	ND	0.065 ± 0.003 [c]	0.111 ± 0.005 [b]	0.128 ±.004[a]

[a-c] Means within a raw with the different superscript are significantly different (p<0.05).

Values are expressed as Mean ± SD. ND = Not Detectable

Table 2: Average total weight (g), length, height (cm) and yield (%) of whole filleting of common carp exposed to m-cresol for 90 days compared to control

	Time of sampling		
		90 day	
Variables	Zero day	Control	m-cresol
Whole body wt. (g)	282 ± 1.2 [b]	300 ± 1.5 [a]	262.1 ± 1.0 [c]
" " length (cm)	18.9 ± 0.1 [b]	19.3 ±0.1 [a]	18.8 ± 0.1 [b]
" " height (cm)	8.3 ± 0.1 [b]	8.7 ± 0.2 [a]	8.2 ± 0.1 [b]
Fillets weight (g)	99.5 ± 0.8 [b]	108.3 ± 0.5 [a]	78.1 ± 0.7 [c]
" length (cm)	10.8 ± 0.1 [b]	11.3 ± 0.2 [a]	10.3 ± 0.1 [c]
" height (cm)	7.4 ± 0.2 [ab]	7.7 ± 0.1 [a]	6.8 ± 0.1 [b]
" yield (%)	35.3 ± 0.2 [b]	36.1 ± 0.3 [a]	29.8 ± 0.3 [c]
Non edible wt. (g)	182.5 ± 0.7 [c]	192.0 ± 0.7 [a]	184.0 ± 0.5 [b]

[a-c] Means within a raw with the different superscript are significantly different (p<0.05).

Values are expressed as Mean ± SD

Table 3: Percentages of moisture, protein, fat, ash and carbohydrate, of common carp fillets following exposure to m-cresol for 90 days compared to control

Parameter	Moisture (%)	Protein (%)*	Fat (%)*	Ash (%)*	Carbohydrate (%)*
Zero day	75.92 ±0.2[ab]	64.16 ± 0.3[b]	24.2 ± 0.1[b]	11.1 ± 0.2[b]	0.44 ± 0.1[b]
90 day Control	75.21± 0.3[b]	66.31 ± 0.4[a]	24.7 ± 0.2[a]	8..3± 0.1[c]	0.69 ±0.02[a]
m-cresol	76.23 ± 0.1[a]	63.52 ± 0.2[c]	24.0 ±0.1[b]	12.07 ± 0.2[a]	0.41±0.2[bc]

[a-c] Means within a column with the different superscript are significantly different (p<0.05).

*(on dry weight). Values are expressed as Mean ± SD

Table 4: Organoleptic scores of common carp following exposure to m-cresol for 90 days compared to control

	Time of sampling		
		90 day	
Variable	Zero day	Control	m-cresol
Appearance	8.0 ± 0.05[b] (V.G.)	9.0 ± 0.06[a] (E.)	7.5 ± 0.07[c] (G.)
Color	9.0 ± 0.07[a] (E.)	9.1 ± 0.05[a] (E.)	7.7 ± 0.06[b] (G.)
Odor	8.5 ± 0.05[a] (V.G.)	8.7 ± 0.04[a] (V.G.)	3.0 ± 0.07[b] (P.F.)
Texture	8.0 ± 0.06[ab] (V.G.)	8.5 ± 0.05[a] (V.G.)	7.0 ± 0.07[b] (G.)
Overall acceptability	83.8 ± 0.7[ab] (V.G.)	88.3 ± 0.8[a] (V.G.)	63.0 ± 0.5[b] (G.)

[a-c] Means within a raw with the different superscript are significantly different (p<0.05).

Values are expressed as Mean ± SD. G. = Good. V.G.= Very good. E = Excellent P.F. = Poorly Fair

The chemical composition (moisture, crude protein, total lipids, ash and carbohydrate %) for muscles of common carp exposed to m-cresol for 90 days were presented in Table 3. The moisture content were approximately 75.92 % for the samples at zero day. Also the moisture was75.21 % and ash was 8.3% in the non-exposed common carp fillets after 90 day. On the other hand, the results showed significant decline in total protein, carbohydrates and lipid contents in the flesh of fish (P<0.05) after 90 day post exposure to m-cresol compared with the zero day and control samples. The highest levels of protein, fat and carbohydrate for control

after 90 day were 66.31, 24.7 and 0.69 %, respectively. Also, moisture and ash reached to 76.23 and 12.07% while, the lowest levels were found for protein (63.52%), fat (24.0%) and carbohydrate (0.41%) in the muscles from treated fish.

Organoleptic Properties: The sensory scores of appearance, color, odor, texture and overall acceptability of common carp exposed to m-cresol were illustrated in Table 4. For control fish, scores after 90 day were 9.0, 9.1, 8.7, 8.5 and 88.3%. in exposed group, scores were 7.5, 7.7, 3.0, 7.0 and 63.0% while for appearance, color, odor, texture and overall acceptability, respectively.

DISCUSSION

The present study showed that long term exposure to m-cresol induced morphological changes in skin of common carp. These effects were attributed to the defensive tissue reactions against direct contact of skin with m-cresol causing its severe irritation and proliferative changes in the epidermis. Also m-cresol causes hypoxia to red cells and hemolysis then hemosiderin pigment release which stimulates proliferation of melanomacrophages which give skin darkening. Moreover the histopathological changes in muscles were attributed to the direct cytotoxic effects of m-cresol on the tissues. Similar lesions were previously mentioned by Alabaster and Lioyd [18], El-Manakhly and Soliman [19], El-Tabakh [6] and Mohamed *et al.* [17].

Regarding residues analysis, the possible explanation for its level through 30, 60 and 90 days PE could be due to long period of exposure which lead to degeneration and necrosis in liver and kidneys and failure of detoxification followed by accumulation of m-cresol in muscles. Andersen [20] reported that m-cresol can be absorbed through skin and the digestive tract; detoxification by the liver and excreted by the kidneys as glucuronide and sulfate metabolites. FAO [21] indicated that, the permissible level of phenolic compounds in fish is 0.01ppm.

The control common carp had higher fillets yield than the fish exposed to m-cresol due to low food consumption during exposure. The actual yield from any individual fish is influenced by a variety of physiological and environmental factors that determine the amount of muscle tissue present in each individual. The achieved data are in agreement with those reported by Souza and Viegas [22]. Also the alterations in chemical composition were attributed to the influences of the toxic effects of m-cresol on physiological and metabolic processes followed by disturbance in the protein, fat and carbohydrate synthesis, lowers the nutritional value of fish then affects fillets yield [22]. These results coincide with those reported by Kaur and Saxena, [26] and Verma and Nath, [27]. They reported a significant decline in the proteins, carbohydrates and total lipid contents in the flesh of fish collected from grossly polluted water. The results were partially similar to those reported by Chatakondi *et al.* [23], Geri *et al.* [24] and Stolle *et al.* [25] they showed that the protein concentration ranged between 13.0 to 21.9%, the lipids content ranged between 0.3 to 23.9%, moisture ranged between 59.8 to 84.2% and ash content ranged between 0.0 to 1.6% in fresh common carp fillets. The deference in the levels were due to a possible correlation to origin, feeding of fish and season.

The result revealed decrease in organoleptic properties which may be attributed to the protein denaturation, hydrolysis and fat oxidation which are the factors influencing the changes in organoleptic properties during m-cresol exposure. Also the results may be attributed to the direct contact of m-cresol to the skin with muscles. Similar results were mentioned by Olah *et al.* [3]. They reported that cresols introduce bad taste and odor to edible flesh of fish. Moreover the cresols had a phenolic odor and caused denaturation of all proteins lead to change in color and texture of muscles [28]. Arnaud *et al.* [29] reported that the rearing conditions affected on the odor of European catfish (*Silurus glanis*) and Varlet *et al.* [30] found that cresols seem to be responsible for the smoked odor in muscles of fish.

Generally it could be concluded that long term exposure to m-cresol affects fish quality may occur through lesions in skin and muscles as well as residues in flesh, also decrease in muscles yield, chemical composition and organoleptic properties. It can be recommended that periodical water analysis should be done for early detection of chemical pollutants as well as the effluents from industries requires further treatment and dilution for the survival of the fish.

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